FACTORS AFFECTING THE ACQUISITION OF *PLAGIORCHIS NOBLEI* (TREMATODA: PLAGIORCHIIDAE) METACERCARIAE BY LARVAE AND PUPAE OF *AEDES AEGYPTI* IN THE LABORATORY

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ABSTRACT. When exposed to concentrations of less than one *Plagiorchis noblei* cercariae per cc of water, the acquisition of metacercariae by *Aedes aegypti* larvae increased significantly with each successive instar but declined precipitously upon pupation. Thus, 1st instar larvae acquired no metacercariae, whereas 4th instars had a mean abundance of 6.15: pupae only acquired a mean of 0.62 parasites. Parasite acquisition was largely a function of host size and activity. Other factors, such as grooming and feeding behavior, may affect the success of cercarial penetration and the distribution of metacercariae in the body of the insect host.

INTRODUCTION

Natural infections with entomophilic digenetic trematodes have been found in a variety of mosquito species, particularly in the genus *Anopheles* (Hall 1929, Timon-David 1958). Furthermore, many authors have reported successful experimental infections of mosquito larvae with stylet-bearing cercariae (xiphidiocercariae) (McMullen 1937, Macy 1960, Dolfus et al. 1960). More recently, laboratory studies revealed that xiphidiocercariae of the genus *Prosthogonimus* will kill *Culex quinquefasciatus* Say larvae (Rao et al. 1985). Similarly, *Aedes aegypti* (Linn.) larvae infected with *Plagiorchis noblei* Park metacercariae exhibit reduced survival and extensive developmental changes (Dempster et al. 1986), and behavioral modifications (Webber et al. 1987a). These changes may render the infected mosquitoes more susceptible to being preyed upon by the definitive host (Webber et al. 1987b).

When insect larvae infected with *P. noblei* are ingested by a vertebrate definitive host, the metacercariae excyst and develop into adult flukes in the intestine. Parasite eggs are passed with the feces of the definitive host and are ingested by lymnaeid snails which serve as the first intermediate host. Within the aquatic snail host, polyembryony gives rise to xiphidiocercariae and, after a brief, free-swimming existence, actively penetrate aquatic insect larvae. Here they encyst to form metacercariae (Blankespoor 1977).

Kavelaars[1] has shown that the acquisition of *Plagiorchis* metacercariae by *Ae. aegypti* larvae increases progressively from the 1st to 4th instar of development. Since the cercariae of *Plagiorchis* are relatively small and disperse slowly (Kavelaars and Bourns 1968), encounters between the parasite and the insect host may be primarily a function of host size and activity. Once contact has been made however, other factors, such as grooming behavior, may influence the success of cercarial penetration. Grooming behavior may also affect the distribution of the metacercariae within the body of the insect host. This study was conducted to gain some insight into the factor(s) that govern the acquisition of *P. noblei* metacercariae by larvae and pupae of *Ae. aegypti.*

MATERIALS AND METHODS

To determine acquisition of *P. noblei* metacercariae by the pre-imago stages of *Ae. aegypti*, 1st, 2nd, 3rd and 4th instar larvae (n = 140, n = 240, n = 140, n = 190, respectively) and pupae (n = 190) were placed in an aquarium (35 x 19.5 x 22 cm) filled to a depth of 20 cm with 13 liters of aerated tap water (20°C). Presoaked tropical fish food (Tetramin, Tetra Co.) was provided as a food source for the larvae. The larvae and pupae were exposed jointly to 9,430 ± 538 ($\bar{x}$ ± SE) freshly emerged cercariae of *P. noblei* obtained from field-collected *Stagnicola elodes* according to the method of Webber et al. (1986). The cercariae exhibit no phototactic responses and remain in suspension for about 12 hours (Bock 1984). After 30 minutes the larvae and pupae were removed from the aquarium and rinsed with aerated tap water to remove loosely adhering cercariae. Larvae, separated according to instar, and pupae were transferred to plastic containers (10.5 cm diam. x 9.0 cm) filled with aerated tap water, provided with food and maintained at 20°C for 3 days. The pre-imagos were then crushed and examined under a compound microscope (40×) to determine the prevalence (percent of larvae infected), mean intensity

[1] Kavelaars, J. 1965. Host-parasite relationships between cercariae of *Plagiorchis noblei* Park and *P. peterborensis* sp.n., and mosquito larvae. M.Sc. Thesis, University of Western Ontario, London, Canada.

(mean number of parasites within infected larvae) and mean abundance (mean number of parasites within all larvae examined, i.e., the product of mean intensity and prevalence) of infection. These terms were used in accordance with the definitions established by Margolis et al. (1982). The data set consisted of two replicates. Homogeneity of variances permitted pooling of replicates. Mean intensity and abundance of infection were compared using a *t*-test (McPair test): prevalence was compared using the Chi-square test (Sokal and Rohlf 1981).

To determine whether differences in parasite acquisition among the various instars are an inherent attribute of their level of development, or whether they are a function of body size, 4th instar larvae of widely divergent sizes were exposed jointly to cercariae of *P. noblei.* Small larvae were produced by rearing them under crowded conditions (1 larva/cc of water, n = 25) whereas larvae of normal size were reared at a density of 1 larva/3.4 cc (n = 24). Both groups were provided with food *ad libitum.* Once larvae attained the 4th instar, they were exposed jointly to 11,982 ± 493 cercariae for 30 minutes. Larvae were then removed from further exposure and maintained in containers as described previously.

Ten randomly selected, uninfected larvae from both the normal and crowded populations were killed in household bleach, transferred to a slide, and measured under a microscope (10×). Body length was measured from the anterior surface of the head to the posterior end of the last anal segment. Body width was measured at the first abdominal segment. The calculation of surface area (mm^2), modified after Kavelaars[1] ($l \times 2\pi r + 2\pi r^2$ where l = length and r = radius), provided a measure of contact between the host and parasite. Three days after exposure infected larvae were crushed and the numbers of metacercariae were recorded. Data were analyzed using analysis of variance (ANOVA). The relationship between infection level and surface area (mm^2) was assessed by linear regression (Sokal and Rohlf 1981).

To determine the distance travelled by the larvae, 10 larvae each of the 2nd and 4th instars were observed in a graduated cylinder (3.5 cm diam. x 25 cm) filled with aerated tap water to 20 cm and provided with food. Each larva was observed for 30 minutes and all vertical movements were measured. Larvae were then measured as described previously. As a mosquito larva travels through a suspension of cercariae in the water column, the predicted number of parasites encountered is contained within a volume of water enclosed by the length and width of the larva and the distance travelled. Second and 4th instar larvae from the same population (n = 43 and 37, respectively) were jointly exposed to a mean of 5,400 ± 301 cercariae in 13 liters of aerated tap water (0.42 cercariae/cc). The predicted number of cercariae encountered by 2nd and 4th instar larvae were calculated (volume × cercarial density) and compared with the actual number of metacercariae acquired. The prevalence and mean intensity and abundance of infection were then calculated for both instars.

To determine whether the distribution of metacercariae between the head, thorax, and abdomen of mosquito larvae is a function of the relative size of these body regions, ten randomly selected 2nd, 3rd, and 4th instar larvae were measured. Length and width of each region were recorded. Then 100 larvae of each instar were exposed jointly to 10,615 ± 476 cercariae of *P. noblei* for 30 minutes and then transferred to separate containers. The number of metacercariae in each body region was determined 3 days later. A Chi-square test compared the observed partitioning of metacercariae between the head, thorax, and abdomen with the distribution of the parasite as predicted on the basis of size (length × width) of these regions. Such a comparison was carried out for each susceptible instar (2nd–4th).

Since the distribution of metacercariae within the population of mosquito larvae was overdispersed, data were normalized by square-root transformation (Sokal and Rohlf 1981). Adjusted means and 95% confidence limits are reported. Distances travelled, lengths, and widths are given as $\bar{x} \pm SE$.

RESULTS

None of the 1st instar larvae of *Ae. aegypti* acquired *P. noblei* infections. Second, 3rd and 4th instar larvae acquired a mean abundance of 0.54, 2.71 and 6.15 metacercariae, respectively (Table 1). Successive instars bore significantly larger numbers of the parasite ($P < 0.05$). Pupae acquired a mean abundance of only 0.62 metacercariae, which differed significantly from that of the 4th instar ($P < 0.05$).

Normal fourth instar larvae were significantly longer and wider (7.71 ± 0.10 mm and 0.89 ± 0.01 mm, respectively) than crowded fourth instar larvae (5.65 ± 0.11 mm and 0.63 ± 0.02 mm, respectively, $P < 0.05$). Normal larvae contained significantly larger numbers of metacercariae than did stunted individuals [$\bar{x} = 5.42$; 95% C.L. = 3.72 − 7.45) and $\bar{x} = 1.10$; 95% C.L. = 0.55 − 1.84, respectively). Regression of larva size (surface area) against level of infection was significant ($P < 0.001$, F = 26.464, regression coefficient = 0.432, y-intercept = −3.907).

Fourth instar larvae travelled significantly

Table 1. Prevalence, intensity and abundance of *Plagiorchis noblei* metacercariae found in the preimago stages of *Aedes aegypti*.

Stage	n	Prevalence (%)	Intensity	Abundance
1st	140	0	0	0
2nd	240	21.2	2.54 (2.39, 2.71)[1]	0.54 (0.46, 0.63)
3rd	140	73.6	4.24 (3.79, 4.72)	2.71 (2.65, 2.72)
4th	190	93.2	6.91 (6.30, 7.56)	6.15 (5.47, 6.81)
Pupa	190	26.3	2.65 (2.45, 2.86)	0.62 (0.52, 0.74)

[1] 95% confidence limits.

Table 2. Observed and predicted distributions of *Plagiorchis noblei* metacercariae in *Aedes aegypti* larvae.

Instar	Body region	Area (mm^2)	No. of metacercariae: Observed	No. of metacercariae: Predicted
2nd	Head	0.43	11	16
	Thorax	0.53	17	20
	Abdomen	1.74	72	64
3rd	Head	0.84	58	43*
	Thorax	1.03	114	55*
	Abdomen	3.67	117	191*
4th	Head	0.97	167	88*
	Thorax	1.44	147	130
	Abdomen	4.94	350	446*

* Observed and predicted values are significantly different at the $P = 0.05$ level.

greater distances in the water column than did 2nd instars (451.8 ± 22.8 cm and 212.7 ± 34.2 cm, respectively, $P < 0.05$) and were significantly larger in size (body length × width) than the latter (4.26 ± 0.26 mm^2 and 0.69 ± 0.05 mm^2, respectively, $P < 0.05$). Thus, 2nd instar larvae travelled through 1.47 cc, whereas 4th instar larvae travelled through 19.30 cc. The predicted number of cercariae encountered by 2nd and 4th instar larvae, based on the above volumes and the density of cercariae (0.42/cc), was 0.61 and 8.11 cercariae, respectively. The actual number acquired (mean abundance) was 0.49 (95% C.L. = 0.32 − 0.68) for 2nd instar larvae and 8.12 (95% C.L. = 7.95 − 8.29) for 4th instars.

Although *Ae. aegypti* larvae grew significantly with each successive instar, the relative size (length × width) of the head, thorax and abdomen of 2nd, 3rd and 4th instar larvae did not differ significantly ($P > 0.05$) (Table 2). These findings are consistent with those reported by Christophers (1960). For 2nd instars, the distribution of metacercariae among the three body regions conformed with their relative size ($P < 0.05$) (Table 2). In contrast, 3rd and 4th instar larvae exhibited fewer metacercariae than predicted in the abdomen, and more than predicted in the head. Levels of infection in the thorax showed no consistent relationship with area (Table 2).

DISCUSSION

The various larval instars and pupae of *Ae. aegypti* differed in their susceptibility to infection with metacercariae of *P. noblei*. Thus, 1st instar larvae were never found to be infected. Thereafter susceptibility increased with each successive instar, confirming the findings of Kavelaars.[1] Susceptibility declined abruptly upon pupation.

Exposure of 4th instar larvae of varying sizes suggests that susceptibility to infection is not an inherent attribute of the level of development. Rather, it is, at least in part, a function of the body size (surface area) of the host. Host size (surface area) alone accounted for ca. 38% of the difference in the observed acquisition of cercariae by 2nd and 4th instar larvae. However, size (body length × width) in conjunction with distance travelled accounted for ca. 80% of observed parasite acquisition. A comparison of the predicted number of cercariae encountered with the actual number of metacercariae acquired confirms this relationship. Fourth instar larvae theoretically encountered 8.11 cercariae and acquired 8.12 metacercariae. Similarly second instar larvae theoretically encountered 0.61 cercariae in their travels and acquired 0.49 metacercariae. Thus, virtually all cercariae encountered by the mosquito larvae gain entrance.

Other factors that may influence the level of infection include parasite-associated changes in host behavior, avoidance reactions, grooming responses and feeding behavior. Webber et al. (1987a) have presented evidence that 3rd instar larvae become more active in the presence of cercariae. Whereas light infections cause a further increase in activity, heavy infections precipitate a decline. Since 2nd instars are only one-sixth the size of 4th instar larvae, even light infections may conceivably cause a reduction in activity in the former, while comparable infections in the latter may result in an increase in activity. Both responses may contribute to the observed difference in infection between 2nd and 4th instar larvae. The relative size of cercariae and larvae of various instars may account for the absence of infection in 1st instar larvae and the low levels in 2nd instar individuals. Such small larvae actively avoid the relatively large cercariae.

Once cercariae have attached themselves to

the cuticle of the larvae, grooming responses may dislodge penetrating parasites. However, as observed by Rees (1952) with chironomid larvae, the effectiveness of these manoeuvres is limited by the inability of the larva to reach its entire body with its mouthparts. An assessment of the distribution of *P. noblei* metacercariae between the head, thorax and abdomen in various instars suggests that grooming responses may be effective in 3rd and 4th stage larvae in reducing parasite penetration in the abdominal regions. Thus, the head of both 3rd and 4th instar larvae and the thorax of 3rd instar larvae harbor significantly more metacercariae than predicted on the basis of area (length × width). This may be attributable to the periodic feeding activity of larvae during which they propel themselves head forward along the bottom of the aquarium by means of their labial brushes. The relatively low susceptibility of pupae to *P. noblei* infection may reflect their low activity and thick cuticle (Christophers 1960).

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Professor R. T. Cronin of Concordia University, Montreal for his generosity in supplying *Aedes aegypti* eggs. Research at the Institute of Parasitology is funded by the Natural Sciences and Engineering Research Council of Canada and les Fonds pour la formation de chercheurs et aide à la recherche (FCAR).

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